

ATTACHMENT B

Amendments to the Specification

Please replace the paragraph at page 28, lines 4-8 with the following amended paragraph:

Each of the compounds was also tested, using an ELISA test, for its ability to compete with the interaction between Grb2 and a phosphotyrosine peptide from the protein Shc and corresponding to the tyrosine 317 (PSPYVNVQN) (SEQ ID N° 5) the affinity of which for Grb2 was evaluated by fluorescence ($K_d = 18 \text{ nM}$).

Please replace the paragraph at page 28, lines 12 through page 5, line 2 with the following amended paragraph:

Plates pre-treated with streptavidine (Boehringer) are incubated overnight at 4°C with 100 µl of a solution of peptide biotin-Aha-PSPYVNVQN (Aha : 6 amino-hexanoic acid) (100 nM solution in TBS buffer : Tris 100 mM, NaCl 50 mM, pH 7.5) per well. The non-specific binding is then blocked by incubating for 4 hours with the same buffer containing 3% of skimmed milk (800 µl per well). The products to be tested are distributed among the wells at the desired dilution in the abovementioned buffer containing 3% of skimmed milk and 40 ng/ml of GST-Grb2 (100 µl per well). After one night's incubation, the plates are carefully rinsed 4 times using TBS-milk-0.05% tween 20, then incubated for 2 hours at 37°C, in the presence of 100 µl per well of anti-GST antibody (transduction, dilution 1/500 in TBS-milk-0.05% tween 20). The plates are then carefully rinsed 4 times using TBS-milk-0.05% tween 20, then incubated for 45 minutes at 37°C in the presence of 100 µl per well of anti-mouse antibody coupled with peroxidase (Amersham, 1/1000 dilution in TBS-milk-0.05% tween 20). The plates

are carefully rinsed using TBS-milk-0.05% tween 20, then incubated in the presence of 200 µl per well of TMB developing solution (Interchim), until a sufficient blue coloration has developed. The reaction is then stopped by adding 100 µl of 10% (V/V) sulphuric acid per well. The reading is taken at 550 nm. The value read off for each well is then reduced by the control value of an equivalent well with no fixed peptide

biotin-Aha-PSpYVNVQN (SEQ ID N°5) then treated, like its homologue, with fixed peptide. The data is then processed using "Origin 40" software to obtain the 50% inhibitory concentrations.